
Myocardial Release of Lactate, Inosine and Hypoxanthine During Atrial Pacing and Exercise-Induced Angina

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SUMMARY The coronary venous efflux of lactate, inosine and hypoxanthine during pacing-induced angina has been compared with myocardial extraction of the catabolites during exercise-induced angina. Inosine and hypoxanthine were analyzed by enzyme assay after separation by column chromatography.

Myocardial lactate extraction at rest (15 ± 9%, mean ± SD) was converted to production levels (−34 ± 26%) during pacing-induced angina (p < 0.0005) and increased (24 ± 13%) during exercise (p < 0.05). The arterial values at rest (850 ± 330 μmol/l) were unchanged during pacing and increased five-fold during exercise (4380 ± 1860 μmol/l). The mean myocardial inosine extraction at rest (33 ± 10%) was transformed to release values (−41 ± 30%) during pacing (p < 0.0005) as well as during exercise (−20 ± 27%) (p < 0.0005). The hypoxanthine extraction at rest (25 ± 11%) decreased during pacing (−7.8 ± 29%) (p < 0.0025) and exercise (10 ± 25%) (NS). The slight increase of arterial inosine and hypoxanthine values was not significant.

Myocardially produced lactate, a sensitive marker of pacing-induced ischemia, was obscured by elevated arterial concentrations during exercise. However, inosine significantly correlated with lactate during pacing, and was useful in detecting ischemic myocardial energy deficiency during exercise-induced angina.

MYOCARDIAL ISCHEMIA leads to anaerobic glycolysis with lactate release from the myocardium, in contrast to lactate utilization by the normally oxygenated heart.\(^1\)\(^-\)\(^9\) Lactate production, reported very frequently during pacing-induced angina, cannot be demonstrated during exercise-induced angina, since metabolic evidence of ischemia is obscured by elevated arterial lactate concentrations.\(^10\)\(^-\)\(^12\) In relationship to anaerobic glycolysis, a decrease of myocardial tissue content of high-energy phosphates, ATP and CP, has been demonstrated.\(^13\)\(^-\)\(^15\) An increase in myocardial tissue content\(^16\)\(^-\)\(^17\) was found and a coronary venous release of the diffusible ATP catabolites adenosine, inosine and hypoxanthine, have been shown\(^19\)\(^-\)\(^23\) in animals after temporary coronary occlusion.
The myocardial release of adenosine and hypoxanthine during pacing-induced angina, as well as our own previous investigations concerning myocardial inosine and hypoxanthine extraction, has been reported also in humans. In this study (preliminary data has been reported) we compare the coronary venous efflux of lactate, inosine and hypoxanthine during pacing-induced angina with myocardial extraction of the catabolites during exercise-induced angina.

Methods

Experimental Procedure

Experiments were performed in 10 patients with stable exercise-induced angina and angiographically proven one-, two- or three-vessel disease. Each patient had 90% stenosis of the left anterior descending branch. Coronary venous blood was obtained through a pacing catheter positioned in the distal portion of the great cardiac vein so blood could be drained from the anterior and septal areas of the left ventricle. A cannula was inserted into the brachial artery for blood sampling. The paced heart rate was increased after 2-minute periods in steps of 10 beats/min until anginal pain of tolerable intensity appeared and continued for 5 minutes. Blood was sampled at rest and another sample was drawn 3–5 minutes after the onset of pacing-induced angina. After a 30-minute recovery period, supine leg exercise was performed. The resistance during exercise was increased from 50 W at the onset in steps of 25 W after 2 minutes exertional periods, until anginal pain occurred. Blood was drawn 1 minute after the onset of angina.

For ECG recordings leads I–III and Wilson leads V₄₆ were used. When a pacing-induced second degree atrioventricular (AV) block occurred, 0.5 mg atropine was administered intravenously to improve AV conduction.

Biochemical Determinations

Lactate was measured enzymatically by conversion to pyruvate with lactate dehydrogenase and NAD. The assay of the ATP catabolites inosine and hypoxanthine was performed by the following analytical techniques, previously described in detail.

Samples of 10 ml coronary venous and arterial blood were drawn into tubes with 10 ml ice-cold solution of heparinized 0.9% NaCl. After centrifugation the diluted plasma samples were ultrafiltered while deproteinization by perchloric acid led to a loss of the purines (mean > 30%). The urine purines were adsorbed onto charcoal and eluted by celite gel filtration with 100 ml of 50% ethanol and 10% pyridine. The evaporated and redissolved filtrates were placed on Sephadex G-10 columns and eluted with water. The urine derivatives were separated by labeled urine compounds [³⁴C]-inosine and [³⁴C]-hypoxanthine (NEN Chemicals) which had been initially added to the plasma samples. The eluates were evaporated, redissolved in 0.5 ml buffer solution and quantitated by the spectrophotometric enzymatic assay of Kalckar.

Recovery Studies

[¹⁴C]-inosine and [¹⁴C]-hypoxanthine with a high specific activity (500 µCi/µmol and 50 µCi/µmol, respectively) were added to the diluted plasma samples. The mean ± SD recovery was 49 ± 8.6% for inosine and 70 ± 10% for hypoxanthine. The radioactivity was measured by a β scintillation counter. A correction for true plasma levels was necessary due to the wide recovery range which was caused by the complex assay.

Calculations

In standard curves, constructed with known concentrations of the nonlabeled compounds inosine and hypoxanthine, the absorbance change of 0.001 was due to 80 nmol/1 inosine and 80 nmol/1 hypoxanthine. The plasma concentrations of inosine and hypoxanthine reported as nmol/1 blood in the unknown solutions were determined using the absorbancies obtained with standard solutions of both purine derivatives (1–50 µmol/1).

The plasma values were calculated in the following equation: plasma concentration of inosine or hypoxanthine (nmol/1 blood) = ([plasma concentration in the unknown solution] × (reaction volume)) ÷ ([recovery of added radioactivity] × (total blood volume)). The lowest measurable value with a confidence range of 99.5%, calculated from the mean difference of the blank value ± 3 SD (= ± 0.003 absorbance change) was 24 nmol/1 (inosine) and 17 nmol/1 (hypoxanthine). The precision of the measurements, calculated from a series of 10 plasma samples, was 5.7% for inosine and 4.8% for hypoxanthine. Myocardial substrate extraction (uptake) values and production (release) values (%) were calculated in the following manner: (arteriovenous difference of substrate concentration ÷ arterial concentration) × 100.

Statistical Methods

The values are given in mean ± 1 SD. The paired t test was used to calculate p values for individual comparison with control and correlations.

Results

Angina was induced by a mean paced heart rate of 141 ± 21 beats/min, with concomitant ST-segment depression of 0.21 ± 0.07 mV in 10 patients with coronary artery disease.

At a mean resistance of 115 ± 35 W during supine leg exercise, performed after a 30-minute recovery period, anginal pain was precipitated in all patients with concomitant ST-segment depression of 0.18 ± 0.13 mV. Coronary venous lactate at rest (720 ± 285 µmol/1) rose during pacing-induced angina (1185 ± 340 µmol/1) (p < 0.0025) and increased fivefold during exercise (3350 ± 1625 µmol/1) (p < 0.0005). The arterial values at rest (850 ± 330 µmol/1) remained unchanged during atrial pacing (905 ± 280 µmol/1) and
increased fivefold during exercise (4380 ± 1860 μmol/l) (p < 0.0005) (fig. 1, top). Myocardial lactate extraction at rest (15 ± 9%) converted to production levels (−34 ± 26%) during pacing (p < 0.0005), while the extraction values increased during exercise (24 ± 13%) (p < 0.05) (fig. 1, bottom).

Coronary venous inosine at rest (575 ± 225 nmol/l) increased during pacing (1510 ± 945 nmol/l) (p < 0.005) as well as during exercise (1120 ± 620 nmol/l (p < 0.01).

The mean arterial value at rest (885 ± 380 nmol/l) showed a slight increase during pacing (1065 ± 635 nmol/l) (NS) and exercise (965 ± 565 nmol/l) (NS) (fig. 2, top, table 1).

The mean myocardial inosine extraction at rest (33 ± 10%) converted to release values (−41 ± 30%) during pacing (p < 0.0005) and exercise (−20 ± 27%) (p < 0.0005) (fig. 2, bottom).

The coronary venous hypoxanthine levels at rest (1180 ± 890 nmol/l) rose during pacing (1640 ± 1075 nmol/l) (NS) and exercise (1995 ± 970 nmol/l) (p < 0.005). The arterial values at rest (1640 ± 1290 nmol/l) remained unchanged during pacing (1620 ± 1120 nmol/l) (NS) (fig. 3, top). The mean hypoxanthine extraction at rest (25 ± 11%) converted to production levels during pacing (−7.8 ± 29%) (p < 0.0025) and decreased during exercise (10 ± 25%) (NS) (fig. 3, bottom).

Discussion

Pacing-induced myocardial lactate release, indicative of anaerobic glycolysis as a consequence of a negative myocardial oxygen balance, has been used as a qualitative marker of myocardial ischemia reflect-
ing the cytoplasmatic NADH + H⁺/NAD ratio. In the normally oxygenated heart pyruvate is catabolized in the citrate cycle. However, during oxygen deficiency with a negative cytoplasmatic and mitochondrial redox potential, increased myocardial NAD in a reduced form is oxidized by a reduction of pyruvate to lactate. To evaluate the changes in lactate extraction to production values, some sources of error must be avoided, as increasing arterial concentrations of lactate cause improved myocardial lactate extraction, which is influenced by the altered extraction ratio of free fatty acids and glucose.

The conversion of myocardial lactate extraction to production levels during angina is accompanied by changes in myocardial inosine and hypoxanthine extraction. Lactate, released from the myocardium during pacing-induced angina, correlates significantly with myocardially produced inosine. Thus, simultaneously released inosine and lactate function as a sensitive marker of myocardial ischemia, since inosine is detecting an ischemic myocardial energy deficiency by the indication of intracellular ATP dephosphorylation and an insufficient glycolytic ATP supply.

ATP and CP breakdown with increased tissue ADP, AMP and purines, as well as intravenously released purines, have been demonstrated after coronary ligation or flow reduction in animals. Coronary venous inosine in pigs increased from 11 to 19 \( \mu \)mol/l and hypoxanthine from 29 to 33 \( \mu \)mol/l with unchanged arterial levels (12 and 28 \( \mu \)mol/l, respectively). However, during ischemia in dogs a coronary venous rise of purine concentrations from low values at rest was more marked and adenosine was detectable as well. The distribution of the ATP-catabolizing enzymes explains the occurrence of ATP catabolites in coronary venous blood. The enzyme 5' nucleotidase histochemically proven in the myocardial cell membrane, affects AMP dephosphorylation, resulting in penetration of adenosine into the interstitial space. Adenosine deaminase, which may be highly concentrated in tissue, plasma and erythrocytes, produces inosine. Nucleoside phosphorylase, localized in the endothelium of coronary vessels, forms hypoxanthine, which is also released from the erythrocytes because of the high erythrocytic nucleoside phosphorylase activity. The lack of xanthine oxidase in human blood inhibits further intracirculatory breakdown to uric acid.

In this study inosine was released from the myocardial extract, indicating the altered extraction of inosine. Figure 2 shows myocardial extraction and production levels of the markers in angina at rest, during pacing- and exercise-induced angina. The paired t test was used to test significance.
Figure 3. (top) Arterial (●) and coronary venous (○) concentrations (nmol/l blood) of hypoxanthine at rest, during pacing- and exercise-induced angina. Positive (−) and negative (−−) arteriovenous differences are shown. (bottom) Myocardial extraction and production levels of hypoxanthine at rest, during pacing- and exercise-induced angina. The paired t test was used to test significance.

During pacing-induced angina. Compared with the uptake levels at rest we calculated a high significance, indicating that the ATP catabolism exceeded the amount of ATP synthesis. As a source of error, the high inosine permeability of human erythrocytes has been avoided by rapid centrifugation and exclusion of hemolysis. Adenosine, the first diffusible ATP catabolite of intact cells, could not be detected because of the enzymatic activity of adenosine deaminase in plasma. The 3H-adenosine which had been added to the plasma samples was converted to 3H-inosine because the radioactivity peak was located in the inosine position on the thin layer radiochromatograms. A conversion to hypoxanthine did not occur, as 3H-hypoxanthine could not be detected. Corresponding to the results reported by Rubio et al., the mean value of adenosine was one-tenth that of inosine and hypoxanthine in dogs after coronary ligature. In further experiments, a short rise in the coronary venous adenosine was followed by a rapid deamination which exceeded the rate of adenosine release. Thus, the additional measurement of adenosine was omitted, since the amount of inosine represents the released adenosine which has been measured after concentration of large blood samples and inactivation of adenosine deaminase. In our study, however, the enzymatic activity in plasma samples was not destroyed by heat because of the high loss of purine compounds (> 30% of labeled standards).

Hypoxanthine extraction, significantly reduced during angina, was not sensitive enough to detect myo-

<table>
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<th>Case no.</th>
<th>At rest</th>
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<th>During exertional angina</th>
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<td>E (%)</td>
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<td>SD</td>
<td>380</td>
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Significance: $p < 0.0005$
cardial ischemia because of the large deviation in the values. However, as a source of error for plasma values, permeation of hypoxantheine out of the erythrocytes has also been avoided by immediate cooling and separation of plasma from erythrocytes after blood sampling. Moreover, the nucleoside phosphorylase activity in plasma was not detectable in the present experiments and the varying increase of the coronary venous hypoxantheine during ischemia may be derived from the low activity of this enzyme in coronary vascular endothelium compared with the proven high adenosine deaminase activity.

Correspondingly, moderately increased hypoxantheine values in cardiac tissue were found after coronary ligation and a small increase of coronary venous hypoxantheine during coronary flow reduction was demonstrated in animals. The usefulness of hypoxantheine as a sensitive marker for myocardial ischemia in humans has been reported. The measured levels of 1–2 μmol/l hypoxantheine are comparable to the present results. These measurements were performed according to the method of Olsson, without preparation and concentration steps, which should be sensitive enough to measure hypoxantheine levels of 90 nmol/l with a highly sensitive spectrophotometer. However, the measurable small absorbance changes below 0.001 are influenced by alterations of sample clarity.

In contrast to simultaneously released inosine and lactate, which is indicative of ischemic myocardial energy deficiency, unchanged inosine extraction before the onset of pacing-induced angina accounts for sufficient ATP production even if glycolysis is demonstrated with lactate release, as correspondingly shown by Parker et al. Thus, simultaneous measurements of lactate and inosine give greater accuracy in the metabolic evaluation of the extent of pacing-induced myocardial ischemia.

The metabolic evidence of ischemia during exercise, however, could not be shown by changes in the myocardial lactate extraction because myocardially produced lactate is obscured by elevated arterial concentrations, with consecutive wide arteriovenous substrate differences. As in the present study, increased lactate extraction to normal levels during exercise has been reported despite the evidence of abnormal left ventricular function. However, significant myocardial inosine release, also during exertional angina, was demonstrated, since arterial inosine values did not alter significantly. Hypoxantheine extraction decreased insignificantly despite a slight increase of arterial levels, which agrees with the findings of Dobson et al. in the ischemic skeletal muscle. However, in the present experiments an increased ATP turnover also in the working not ischemic muscle may explain the slightly increased arterial hypoxantheine values.

Therefore, inosine release indicates that exercise-induced angina is not accompanied by seemingly improved myocardial aerobic metabolism, as it may be derived from unchanged or increased lactate extraction.

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